Amendments to the Specification:

Please replace the paragraph beginning at page 1, line 4, with the following rewritten paragraph:

-- This application is a continuation of~~, and claims the benefit priority from U.S. Patent~~ Application ~~Serial~~ No. 08/446,669, filed January 11, 1995, now U.S. Patent No. 6,132,987, which is the National Stage of International Application No. PCT/US95/00476, filed January 11, 1995, which is a continuation-in-part of Application No. 08/182,962, filed January 13, 1994, now abandoned, the full disclosure of which is incorporated by reference in its entirety. --

Please replace the paragraph beginning on page 4, line 30, with the following rewritten paragraph:

-- Another aspect of this invention is DNA sequences (SEQ ID NO:1 and SEQ ID NO:3) that encode the expression of the MCP-1RA and 1RB proteins. These DNA sequences may include an isolated DNA sequence that encodes the expression of a MCP-1R protein as described above. As used here, "isolated" means substantially free from other mammalian DNA or protein sequences with which the subject DNA or protein sequence is typically found in its native, i.e., endogenous, state. The DNA sequences coding for active MCP-1RA and 1RB are characterized as comprising the same or substantially the same nucleotide sequence as in Figures 1 and 2 (SEQ ID NOS: 1 and 3), respectively, or active fragments thereof. The DNA sequences may include 5' and 3' non-coding sequences flanking the coding sequence. The DNA sequences may also encode an amino terminal signal peptide. Figures 1A-1D and 2A-C illustrate the non-coding 5' and 3' flanking sequences and a signal sequence of the MCP-1RA and 1RB sequences, respectively, isolated from the human monocytic cell line MonoMac 6 and expressed in Xenopus oocytes. --

Please replace the paragraph beginning on page 5, line 13, with the following rewritten paragraph:

-- It is understood that the DNA sequences of this invention may exclude some or all of these signal and/or flanking sequences. In addition, the DNA sequences of the present invention

encoding a biologically active human MCP-1R protein may also comprise DNA capable of hybridizing under appropriate stringency conditions, or which would be capable of hybridizing under such conditions but for the degeneracy of the genetic code, to an isolated DNA sequence of ~~Figure~~ Figures 1A-1D or ~~Figure~~ Figures 2A-2C (SEQ ID NOS:1 and 3). Accordingly, the DNA sequences of this invention may contain modifications in the non-coding sequences, signal sequences or coding sequences, based on allelic variation, species variation or deliberate modification. Additionally, analogs of MCP-1R are provided and include truncated polypeptides, e.g., mutants in which there are variations in the amino acid sequence that retain biological activity, as defined below, and preferably have a homology of at least 80%, more preferably 90%, and most preferably 95%, with the corresponding region of the MCP-1R sequences of ~~Figure~~ Figures 1 A-1D or ~~Figure~~ Figures 2 A-2C (SEQ ID NOS: 2 and 4). Examples include polypeptides with minor amino acid variations from the native amino acid sequences of MCP-1R of ~~Figure~~ Figures 1A-1D and 2A-2C (SEQ ID NOS: 2 and 4); in particular, conservative amino acid replacements. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into four families: (1) acidic = aspartate, glutamate; (2) basic = lysine, arginine, histidine; (3) non-polar = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar = glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid will not have a major effect on activity or functionality.--

Please replace the paragraph beginning on page 6, line 10, with the following rewritten paragraph:

--Using the sequences of ~~Figure~~ Figures 1 A-1D and ~~Figure~~ Figures 2A-2C (SEQ ID NOS: 1, 2, 3 and 4) as well as the denoted characteristics of a MCP-1R receptor molecule in general, it is within the skill in the art to obtain other polypeptides or other DNA sequences encoding MCP-

1R. For example, the structural gene can be manipulated by varying individual nucleotides, while retaining the correct amino acid(s), or varying the nucleotides, so as to modify the amino acids, without loss of activity. Nucleotides can be substituted, inserted, or deleted by known techniques, including, for example, in vitro mutagenesis and primer repair. The structural gene can be truncated at its 3'-terminus and/or its 5'-terminus while retaining its activity. For example, MCP-1RA and MCP-1RB as encoded in ~~Figure~~ Figures 1A-1D and ~~Figure~~ Figures 2A-2C (SEQ ID NOS:1 and 2; SEQ ID NOS:3 and 4) respectively, contain N-terminal regions which it may be desirable to delete. It also may be desirable to remove the region encoding the signal sequence, and/or to replace it with a heterologous sequence. It may also be desirable to ligate a portion of the MCP-1R sequences (SEQ ID NOS: 1 and 3), particularly that which includes the amino terminal domain to a heterologous coding sequence, and thus to create a fusion peptide with the receptor/ligand specificity of MCP-1RA or MCP-1RB.--

Please replace the paragraph beginning at page 9, line 14, with the following rewritten paragraph:

--FIGS. 1A-1D illustrate ~~FIG. 1 illustrates~~ the human cDNA and amino acid sequences (SEQ ID NO:1 and SEQ ID NO:2, respectively) of the isolated MCP-1 receptor clone, MCP-1RA. --

Please replace the paragraph beginning at page 9, line 17, with the following rewritten paragraph:

--FIGS. 2A-2C illustrate ~~FIG. 2 illustrates~~ the human cDNA and amino acid sequences (SEQ ID NO:3 and SEQ ID NO:4, respectively) of the isolated MCP-1 receptor clone, MCP-1RB. --

Please replace the paragraph beginning at page 9, line 20, with the following rewritten paragraph:

--~~FIG. 3 illustrates~~FIGS. 3A and 3B illustrate the results of Northern blot analysis of hematopoietic cell lines that were probed for MCP-1RA and MCP-1RB mRNA, respectively. --

Please replace the paragraph beginning at page 9, line 22, with the following rewritten paragraph:

--~~FIG. 4 illustrates~~ FIGS. 4A and 4B illustrate the predicted amino acid sequence of the MCP-1 receptor A (MCP-1RA)(SEQ ID NO:2), aligned with the MIP-1α/RANTES receptor sequence (SEQ ID NO:5), the orphan receptor sequence HUMSTSR (SEQ ID NO:6) and the two IL-8 receptor sequences (SEQ ID NOS:7 and 8). Identical residues are boxed. The seven putative transmembrane domains are indicated by the horizontal bars. Gaps inserted to optimize the alignments are indicated by dashes. Amino acid numbers for each sequence are located to the right of the sequences. --

Please replace the paragraph beginning at page 10, line 6, with the following rewritten paragraph:

--~~FIG. 7~~ FIGS. 7A and 7B graphically depict the binding of ^{125}I-MCP-1 to the recombinant MCP-1RB receptor, as described in detail in Example 5. --

Please replace the paragraph beginning at page 10, line 8, with the following rewritten paragraph:

-- ~~FIG. 8~~ FIGS. 8A-8C graphically ~~depicts~~ depict the results of the MCP-1RB receptor-mediated calcium mobilization experiments also described in detail in Example 5. FIG. 8A depicts intracellular calcium flux as a function of MCP-1 concentration (nM). Calcium transients peaked at 4-8 sec. after addition of MCP-1 and returned to baseline within 90 sec. of activation. FIG. 8B depicts the MCP-1 stimulated calcium mobilization (EC.sub.50 =3.4 nM) and the lack of

stimulated calcium mobilization by other cytokines. FIG. 8C illustrates that MCP-1 desensitized the cells to a second addition of MCP-1. --

Please replace the paragraph beginning on page 11, line 19, with the following amended paragraph:

--To obtain a full-length version of this clone, an MM6 cDNA library was constructed and probed with the PCR product. An isolated clone of 2.1 kb was obtained and called MCP-1RA. FIGS. 1A-1D illustrate[s] the cDNA sequence (SEQ ID NO: 1) and the predicted amino acid sequence (SEQ ID NO:2) of the clone. The nucleotide sequence (SEQ ID NO:1) comprises 2232 base pairs, including a 5' noncoding sequence of 39 base pairs and a 3' noncoding sequence of 1071 base pairs. The MCP-1RA sequence is characterized by a single long open reading frame encoding a 374 amino acid following the initiation methionine at position 23.--

Please replace the paragraph beginning on page 12, line 3, with the following amended paragraph:

--The predicted amino acid sequence of MCP-1RA (SEQ ID NO:2) reveals seven putative transmembrane domains and an extracellular amino terminus of 40 residues. Further analysis of the MCP-1RA amino acid sequence reveals several interesting features. Despite its homology with the related MIP-1 α /RANTES receptor and the IL-8 receptors, MCP-1RA exhibits significant divergence in its amino and carboxyl termini. See FIGS. 4A-4B (SEQ ID NOS: 2, 5, 6, 7 and 8). Additionally, a striking identity between MCP-1RA and the MIP-1 α/RANTES receptor occurs in a 31 amino acid sequence beginning with the septate IFFIILL at the end of the third transmembrane domain.--

Please replace the paragraph beginning on page 12, line 22, with the following amended paragraph:

--The MCP-1R polypeptides provided herein also include polypeptides encoded by sequences similar to that of MCP-1RA and 1RB (SEQ ID NOS: 1, 2, 3 and 4) in FIGS. 1A-1D and 2A-2C, but into which modifications are naturally provided or deliberately engineered. This invention

also encompasses such novel DNA sequences, which code on expression for MCP-1R polypeptides having specificity for the MCP-1 receptor. These DNA sequences include sequences substantially the same as the DNA sequences (SEQ ID NOS: 1 and 3) of FIGS. 1A-1D and 2A-2C and biologically active fragments thereof, and such sequences that hybridize under stringent hybridization conditions to the DNA sequences (SEQ ID NOS: 1 and 3) of FIGS. 1A-1D and 2A-2C. See Maniatis, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387-389. One example of such stringent conditions is hybridization at 4 .times.SSC, at 65 degrees C., followed by a washing in 0.1 × SSC at 65 degrees C. for one hour. Another exemplary stringent hybridization scheme uses 50% formamide, 4 × SSC at 42 degrees C.--

Please replace the paragraph beginning on page 13, line 5, with the following amended paragraph:

--DNA sequences that code for MCP-1R polypeptides but differ in codon sequence due to the degeneracies inherent in the genetic code are also encompassed by this invention. Allelic variations, i.e., naturally occurring interspecies base changes that may or may not result in amino acid changes, in the MCP-1R DNA sequences (SEQ ID NOS: 1 and 3) of FIGS. 1A-1D and 2A-2C encoding MCP-1R polypeptides having MCP-1R activity (for example, specificity for the MCP-1 receptor) are also included in this invention.--

Please replace the paragraph beginning on page 26, line 12, with the following amended paragraph:

--A method is provided for identifying ligands of the MCP-1 receptor, such as antagonists. The method comprises transfecting a mammalian cell line with an expression vector comprising nucleic acid sequences encoding the N-terminal domain of MCP-1 receptor (see SEQ ID NOS:1 and 3). The N-terminal domain of the MCP-1 receptor may be expressed alone or in combination with other domains of the MCP-1 receptor. The other domains may be extracellular, intracellular or transmembrane domains. Moreover, a chimaeric protein may be expressed, where the other domains are the corresponding domains from related proteins, such as those in ~~Fig.~~ FIGS. 4A

and 4B (SEQ ID NOS:5, 6, 7 and 8). The N-terminal domain may also be expressed as a portion of the native MCP-1 receptor. Expression of extracellular domains is preferred where soluble protein for solid phase assays is required.--

Please replace the paragraph beginning at page 30, line 13, with the following rewritten paragraph:

-- PCR reactions were carried out for 30 cycles beginning with a 1-minute incubation at 94°C, 2 minutes at 50°C, 1.5 minutes at 72°C, and a final elongation step at 72°C for 4 minutes using the PCR primers described above (SEQ ID NOS: 9 and 10) at a final concentration of 1 μM and MM6 cDNA at approximately 10 ng/ml. PCR products migrating between 200-300 base pairs on a 1.5% agarose gel were excised, subcloned into pBluescript (sk) and sequenced using fluorescently labeled dideoxyribonucleotides as described by Sanger, Proc Natl Acad ~~SciUSA~~ Sci USA 74:5463-67(1977). Sequence analysis revealed cDNAs encoding the predicted fragments of the receptors upon which the primers were designed and one cDNA which appeared to encode a novel protein. To obtain a full-length version of this clone, an appropriate cell line was chosen and a cDNA library was constructed in pFROG and probed with this PCR product, as detailed in subsections 3 and 4 below. --

Please replace the paragraph beginning on page 32, line 27, with the following rewritten paragraph:

--The full sequence of MCP-1RA cDNA (SEQ ID NO:1) and the encoded amino acid sequence (SEQ ID NO:2) are shown in FIGS. 1A-1D. The encoded protein sequence is shown below that of the cDNA sequence. The cDNA sequence (SEQ ID NO:3) and encoded amino acid sequence (SEQ ID NO:4) of MCP-1RB are shown in ~~Figure~~ Figures 2A-2C. Conventional numbering is used.--

Please replace the paragraph beginning on page 33, line 1, with the following rewritten paragraph:

--The translation of both MCP-1R DNAs is most likely initiated at the ATG start codon. This is the only in-frame MET codon in the 5' region of the cDNA. Following the initiating methionine (MET) is an open reading frame encoding a protein of 374 amino acids with a predicted molecular weight of about 42,000 Daltons. By direct comparison with the known transmembrane domains for the MIP-1α/RANTES receptor, the orphan receptor HUMSTSR and the IL-8 receptors 8RA and 8RB, an extra cellular amino terminus of 48 residues is revealed. The transmembrane domains are most likely located at amino acids 49 through 70, 80 through 700, 115 through 136, 154 through 178, 204 through 231, 244 through 268 and 295 through 313. They are indicated in FIGS. 4A and 4B by the ~~horizonal~~ horizontal lines above the sequence groupings (SEQ ID NOS: 2, 5, 6, 7 and 8). The carboxyl tail of 61 amino acids begins with serine at position 314 (see FIGS. 4A and 4B).--

Please replace the paragraph beginning on page 33, line 13, with the following rewritten paragraph:

--The MCP-1RB cDNA encodes an amino acid sequence identical to that of MCP-1RA from the MET at position 1 through the arginine at position 313 and including 30 untranslated nucleotides immediately 5' of the initiating MET (see FIGS. 2A-2C). The putative amino acid sequence of MCP-1RB (SEQ ID NO:4) however reveals a completely different cytoplasmic tail than the 61 amino acid cytoplasmic tail of MCP-1RA (SEQ ID NO:2). MCP-1RB has a cytoplasmic tail of 47 amino acids beginning with arginine at amino acid position 314 and ending with leucine at position 360. That alternative splicing occurred at position 313 can be inferred from the sequence identity, including the 5' untranslated sequence, of the two clones and from the characteristic AG sequence located at the putative donor junction between amino acid positions 313-314. In addition, a cDNA common to both A and B forms of MCP-1R hybridized to a single band on Southern blots of human genomic DNA under high stringency conditions, and one cDNA clone from the MM6 library was obtained that contained in tandem both carboxyl-terminal cytoplasmic tails found in MCP-1RA and 1RB, suggesting derivation from incompletely processed RNA. The MCP-1 receptor, MCP-1R, is only the second known example of alternative splicing of the carboxyl tails of receptors in the seven-transmembrane receptor family. Namba, Nature 365:166-

70(1993) has reported that the prostaglandin (PG) E2 receptor has four alternatively spliced carboxyl-terminal tails with little sequence homology among the four. The related MIP-1α/RANTES and IL-8 receptors are believed to be intronless. See Holmes, Science 253:1278-80(1991); Murphy, Science 253:1280-83(1991) and Neote, Cell 72:415-25(1993). Alignment of the cytoplasmic tails of MCP-1RA and 1RB with other chemokine receptors revealed that one of the receptors, MCP-1RB, was homologous to the corresponding region in the MIP-1 α /RANTES receptor. The carboxyl tail of MCP-1RA bore no significant identity with other known proteins.--

Please replace the paragraph beginning on page 34, line 8, with the following rewritten paragraph:

--Northern blots of hematopoietic cell lines were performed as described in Sambrook, Molecular Cloning: A Laboratory Manual, Second Edition (1989), and probed for each of the MCP-1R clones revealed that both mRNA species migrated as a single 3.5 kb band. See FIGS. 3A and 3B. Both mRNAs were expressed at approximately equal levels in the MCP-1 responsive cell lines MM6 and in THP-1 cells. Neither were expressed in the unresponsive cell lines HEL and HL-60. Expression of each of the mRNA was also detected in freshly isolated human monocytes by reverse transcription PCR.--

Please replace the paragraph beginning on page 34, line 20, with the following rewritten paragraph:

--Comparison of the sequences of MCP-1RA (SEQ ID NO:2) with the IL-8 receptors RA and RB, the MIP-1.alpha./RANTES receptor and the orphan receptor HUMSTSR (SEQ ID NOS:7, 8, 5 and 6, respectively) is illustrated in FIGS. 4A-4B. Comparison of the deduced amino acid sequence of the novel MCP-1A receptor with other seven transmembrane proteins revealed that it most closely relates to the MIP-1 α /RANTES receptor, with 51% identity at the amino acid level. The IL-8 receptors RA and RB exhibited 30% identity at the amino acid level to and the HUMSTSR orphan receptor exhibited 31% identity at the amino acid level. Analysis reveals that

the MCP-1 receptor has diverged from the related MIP-1 α /RANTES receptor and the IL-8 receptors in its amino-terminal and carboxyl-terminal domains. A striking identity between the MCP-1A receptor and the MIP-1 α /RANTES receptor is found in the sequence IFFHLLTI DRYLAIV HAVFAL(K/R) ARTVTFGV (SEQ ID NOS: 13 and 14), which occurs at the end of the third transmembrane domain (see FIGS. 4A-4B). The corresponding region of rhodopsin is known to participate in G-protein binding (Franke et al., Science 250:123 (1990)), suggesting that this domain may mediate aspects of G-protein activation common to receptors for C-C chemokines.--

Please replace the paragraph beginning on page 36, line 28, with the following rewritten paragraph:

--Equilibrium binding assays were then performed using the method of Ernst, J. Immunol. 152: 3541-49 (1994). Varying amounts of ^{125} I-labeled MCP-1 (Dupont-NEN, Boston, Mass.) were incubated with 6×10^6 MCP-1RB expressing HEK-293 cells resuspended in binding buffer (50 mM Hepes, pH 7.2, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% BSA (bovine serum albumin, fraction V, Sigma)) in the presence or absence of 100-fold excess of the unlabeled C-C chemokines MIP-1 α, MIP-1 β and RANTES, and the C-X-C chemokine IL-8 (chemokines obtained from R&D Systems, Inc., Minneapolis, Minn). Competition experiments were performed using 500 pM ^{125}I-labeled MCP-1 and the concentrations of unlabeled chemokines as indicated in ~~Fig.~~ FIGS. 7A-7B.--

Please replace the paragraph beginning on page 37, line 7, with the following rewritten paragraph:

--Equilibrium binding data were analyzed according to the method of Scatchard using the program "LIGAND" (Biosoft, Ferguson, Mo.) on a Macintosh computer. See Munson, Anal. Biochem. 107: 220-39 (1980). The closely related C-C chemokines MIP-1α, MIP-1β, and RANTES, as well as the C-X-C chemokine IL-8 did not compete for binding. Nor was specific binding detected in transfectants that expressed little or no MCP-1RB on Northern blots. Analysis of equilibrium binding data shown in ~~Fig.~~ FIGS. 7A and 7B indicates a dissociation

constant (K_d) of 260 pM (~~Fig.~~ FIG 7B). This K_d is in good agreement with that reported for the binding of MCP-1 to monocytes (Yoshimura, J. Immunol. 145:292-97 (1990); Zhang, J. Biol. Chem. 269:15918-24 (1994)) and THP-1 cells (Van Riper, J. Exp. Med. 177:851-56 (1933)). These data indicate that ^{125} I-MCP-1 bound specifically and with high affinity to the MCP-1RB receptor expressed in 293 cells.--

Please replace the paragraph beginning on page 38, line 28, with the following rewritten paragraph:

--MCP-1 stimulated robust calcium mobilization in the stably transfected MCP-1RB/293 cells in a specific and dose-dependent manner. Small but reproducible signals were seen with as little as 100 pM MCP-1, and the average EC_{50} from four full dose-response curves to MCP-1 was 3.4 nM (2.7-4.4 nM; ~~Fig.~~ FIGS [8, *A* and *B*] 8A and 8B). The MCP-1RB receptor was selectively activated by MCP-1. RANTES, MIP-1α, MIP-1β, Gro- α, and IL-8 failed to stimulate significant calcium signals in these same cells, even when present at high concentrations (Fig. 8*B*). Furthermore, these chemokines also failed to block stimulation of the cells by MCP-1, indicating that they are unlikely to act as endogenous antagonists of the MCP-1RB receptor. The MCP-1-dependent intracellular calcium fluxes were characterized by short lag times, followed by a rapid rise in $[Ca^{2+}]_i$ that returned to near basal levels within 80-90 sec of the addition of MCP-1 (Fig. 8*A*). The cells demonstrated homologous desensitization in that they were refractory to activation by a second challenge with MCP-1 (Fig. 8*C*).--

Please replace the paragraph beginning on page 45, line 6, with the following rewritten paragraph:

--MCP-1RB was remarkably specific for MCP-1. In the cyclase assay the IC_{50} for inhibition by MCP-1 was 90 pM, whereas related chemokines were ineffective at up to 1 μM. In contrast, the MIP-1 α/RANTES ~~MCP-1-α/RANTES~~ receptor has an IC50 of approximately 100 pM for MIP-1 α and RANTES, and 10 nM and 820 nM for MIP-1β and MCP-1, respectively. Thus, MCP-1 had a selectivity of at least 9000-fold for the MCP-1 receptor, whereas MIP-1 α and RANTES had a similar preference for the MIP-1 α/RANTES ~~MCP-1-α/RANTES~~ receptor, as compared to

MCP-1RB. It is likely, therefore, that under physiological conditions, MCP-1, MIP-1 α, and RANTES act as specific agonists of MCP-1RB and the MIP-1 α/RANTES ~~MCP-1-α/RANTES~~ receptor, respectively.--

Please replace the paragraph beginning on page 45, line 30, with the following rewritten paragraph:

--MCP-1 is synthesized and secreted in vitro by a number of different cells in response to a variety of different cytokines or oxidatively modified lipoproteins. The specificity of the cloned receptor for MCP-1, coupled with the fact that only monocytes, basophils, and a subset of T lymphocytes response to MCP-1, provides for an effective means of limiting the spectrum of infiltrating leukocytes in areas where MCP-1 is abundant. Early atherosclerotic lesions have a predominantly monocytic infiltrate and MCP-1 is abundant in these lesions. In contrast, the MIP-1 α/RANTES ~~MCP-1-α/RANTES~~ receptor binds and signals in response to multiple chemokines, and may serve to mediate more complex inflammatory reactions. Once activated, however, the MCP-1 and MIP-1 α/RANTES ~~MCP-1-α/RANTES~~ receptors appear to use similar signal transduction pathways.--